EXPERIMENTAL METHODS FOR THE STUDY OF PROTEIN MONOLAYERS

by

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April, 1946.

A THESIS

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FOR
THE STUDY OF PROTEIN MONOLAYERS

Submitted in Partial Fulfillment
of the
Requirements for the degree of
Master of Science

by

J. W. V. Hahn, B.Sc.

Under the direction of
Dr. J. L. Morrison

Time Devoted to this Work - 19 Months

Edmonton, Alberta. April, 1946.

The author wishes to express his appreciation to all who have assisted in the completion of this work:

To Dr. J. L. Morrison, under whose guidance this project was undertaken, for his constant interest and assistance during the course of the investigation.

To Dr. H. E. Johns who assisted in the design of the electrical apparatus.

To Mr. W. A. Butcher who constructed the horizontal film balance and contributed many helpful suggestions on its design.

To the Department of Chemistry for the provision of laboratory facilities and materials.

To the Committee on Agricultural Research Grants who made it financially possible for the project to be undertaken.

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INTRODUCTION

This work is part of a general project to investigate the properties of plant proteins. The great variability in the nature of plant protein requires that its properties be attacked simultaneously by many research tools. This particular work deals with the development of apparatus to study the plant proteins when spread on water surfaces. By means of this apparatus it is hoped to measure the molecular weights and the electrical and mechanical characteristics of spread protein molecules.

When a substance spreads on a liquid surface to a thickness of one molecule, it is called a monomolecular film.

Monomolecular films (or monolayers) are a most interesting state of matter. In them the molecules are often arranged in a very simple manner, so that by a study of the films much can be learned as to the size, shape and form of the molecules. Monolayers may exist in many different forms, which correspond, in the two-dimensions of the surface to the three principal states of matter in the three dimensions, solid, liquid and gaseous. The principal factors deciding the state of the surface films are the amount and distribution of the adhesive forces between the molecules laterally. Monolayers have measurable effects on the mechanical, electrical and optical properties of the surface.

For the investigation of the properties of an monolayers two methods are used.

(1) Determination of the loss in surface tension which the layers being used by reason of a film balance.

(2) Determination of the change in surface potential which the layers produce.

These methods are continued supplemented by studies of

(a) expansion patterns involving the use of an "indicator oil".

(b) the viscosity of the mixed films at varying pressures.

(c) optical effects produced by change in the structure of the film.

THEORY OF MONOLAYERS.

Spread Monomolecular Layers.

When a small amount of an insoluble substance is placed on a clean water surface, one of two things may happen. The substance may remain as a compact mass, leaving the rest of the surface clear, or it may spread out over the surface. If the energy requirements for spreading are fulfilled the substance will spread over the whole of the surface having a film one molecule thick. If the area is not large enough to accommodate all of the substance as a monolayer the final state of the surface is one in which the greater part is covered by a monolayer, the excess of the substance being present as a lens or lenses.

There are three theories with respect to spreading (1), (2).

(1) Lord Rayleigh's theory in which it is considered that all liquids spread on water and that lenses of a liquid do not form on water unless the surface of the water is contaminated by some foreign substance.

(2) Langmuir's theory which states: Liquids whose molecules contain polar groups spread on water; if polar groups are not present the liquid will not spread. Thus: "The only substances which spread are those whose molecules contain both hydrophilic (water-loving) and hydrophobic (water-hating) parts."

(3) Harkin's theory, a general thermodynamic theory which may be applied to all liquids, to the spreading

of mobile films on solids. The theory is based on the concept that there are two types of spreading:

(A) Duplex or D spreading.

(B) Non-duplex or N spreading.

A duplex film is defined as a film so thin as to have properties which are practically independent of the force of gravitation, but which is sufficiently thick to allow the interfacial energy, both free and total, of each of its interfaces to be the same as when both liquids are present in the bulk, so that each interface is independent of the other. A non-duplex film may be spread directly from a three-dimensional liquid or solid, or it may be formed by the transformation of a duplex film. All non-duplex oil films on water, whose structures are known are monolayers.

Duplex spreading: The theory of the spreading of a duplex film may be stated in the following way: If a drop of an insoluble oil (b) is placed on a clean water (a) surface it may or may not spread. If the oil films itself (b) better than water (a) it will not spread, while if it likes the water better than itself, it will spread. Expressed quantitatively:

$$S_{b/a} = -\left(\frac{\partial F}{\partial \sigma}\right)_{p,T} = W_A - W_{cb}$$

where, $-\left(\frac{\partial F}{\partial \sigma}\right)_{p,T}$ is the rate of decrease of the free energy (F) of the system with an increase of area (σ) of the duplex film. W_A is the work required to pull the oil and water apart over an area of 1 sq. cm. and W_{cb} is that required to replace a box of oil with a cross section of 1 sq. cm. A consideration of these processes gives values for W_A and W_{cb} as follows.

$W_A = \gamma_a + \gamma_b - \gamma_{ab}$, $W_{cb} = 2\gamma_b$

so, $S_{b/a} = \gamma_a - (\gamma_b + \gamma_{ab})$

Application of the equation to benzene results in the following value for $S_{b/a}$, the initial spreading coefficient:

$$S_{b/a} = \gamma_a - (\gamma_b + \gamma_{ab}) = 72.8 - (28.9 + 35.0) = 8.9 \text{ ergs/cm}^2.$$

Thus an increase of 8.9 ergs in the free energy results for an increase of 1 sq. cm. in the area of the duplex film. This indicates that benzene should spread rapidly to form a duplex film.

However, since the extremely thin duplex film of benzene becomes saturated with polar type soaps, the coefficient of spreading, which may be designated the semi-initial coefficient, becomes

$$S_{b'/a} = \gamma_a - (\gamma_{b'} + \gamma_{a'b'}) = 72.8 - (28.8 + 35.0) = 8.8 \text{ ergs/cm.}^2$$

where the prime indicates that b is saturated with a. The value of this coefficient indicates that benzene also spreads on a clean water surface to form a duplex film.

If b and a are mutually saturated, then

$$S_{b'/a'} = \gamma_{a'} - (\gamma_{b'} + \gamma_{a'b'}) = 62.2 - (28.8 + 35.0) = -1.6 \text{ ergs/cm}^2$$

This may be designated as the final spreading coefficient. The negative value for the final spreading coefficient indicates that benzene will not spread over the surface of water if the liquids are mutually saturated. The small change between the semi-initial and final coefficient of spreading is due to a decrease of 10.6 ergs, per sq. cm. in the free surface energy of the water surface.

Now if the free surface energy of the mutually saturated system is subtracted from the free surface energy of the clean water system, there is obtained as a difference (7le)

$$\pi_e = \gamma_w - \gamma_e = \gamma_a - \gamma_{a'} = S_{b/a} - S_{b/a'}$$

where π_e is by definition the film pressure, the subscript in π_e and γ_e indicates that the film of the oil b is in equilibrium with a layer of the oil. Thus a difference in the two spreading coefficients yields a result equal to the film pressure for a non-duplex film of benzene on the water. Experiment confirms this value and values obtained by similar calculation for other substances such as carbon disulfide and methylene iodide. For the substances mentioned it would appear that the final coefficient of spreading is negative in all cases and hence the result is to form more stable than the duplex film. Thin oriented oily and fatty polymolecular oil films on water have not been found to exist for any considerable time. These theories therefore indicate that liquids with a positive initial spreading coefficient may spread on water to form duplex films, and that these films are themselves intermolecular.

It would appear that polar groups are not necessary for spreading, as, for instance,

$$-\Delta F = -\gamma_a + \gamma_b + \gamma_{ab}$$

for unit area; the presence of a hydrophilic group such as $-COOH$, $-OH$, $-NH_2$ lowers γ_a and so is promoting. The presence of a hydrophilic group is sometimes found to make them soluble. Hydrocarbons are characteristically insoluble in water. If groups such as $-COOH$, $-OH$, $-NH_2$, and $-O-$ are substituted into a hydrocarbon they tend to make it water soluble. The hydrocarbon portion is hydrophobic. If the hydrocarbon chain is small as the

A substance dissolved in a liquid will do one of two things to the surface tension of the liquid: it will either raise or lower it (1). If the addition of the solute raises the surface tension, it is said to be capillary inactive; if it lowers the surface tension, the solute is capillary active. Most inorganic electrolytes in appreciable concentrations are capillary inactive. Some proteins, and a great many other organic compounds are capillary active. Capillary active substances are positively adsorbed at the surface of the liquid, that is, the concentration of the dissolved substance is higher at a surface than in the bulk of the solution. The relation between the change in surface tension of a solution and the amount of solute adsorbed at the surface is given by Gibbs' adsorption equation: $\frac{d\gamma}{dc} = -\frac{a}{c}\frac{dP}{dc}$

in which a is the concentration of the solute at the surface in excess of that in the solution, c is the bulk concentration of the solute, $\frac{d\gamma}{dc}$ is the rate of change of the surface tension with concentration, and $\frac{dP}{dc}$ is the rate of change of the osmotic pressure of the solute with concentration. If the ideal gas laws are assumed to apply to the osmotic pressure of the solute, that is, if

$$P = CRT \qquad \frac{dP}{dc} = RT$$

then Gibbs' equation becomes $a = -\frac{c}{RT}\frac{d\gamma}{dc}$

Using the Gibbs' equation and values of $\frac{d\gamma}{dc}$ obtained from an empirical equation proposed by Szyszkowski to describe the surface tension lowering of a solution containing a

capillary active solute, Langmuir (4) was able to show that the short chain fatty acid molecules in concentrated solutions were orientated to the surface when adsorbed at the surface.

Thus it can be seen that the surface tension of water is lowered both by spread monomolecular films of insoluble substances and by the presence of surface active or capillary active soluble substances dissolved in the water.

THE MECHANISM OF SPREADING OF LIQUIDS

The mechanism of spreading of insoluble substances on water is discussed by Adam [1]. The motions of the water molecules cause the expanding movement of the oil drop. The molecules of water are in constant motion parallel to the surface, diffusing to long distances. The oil molecules adhere to them, and are carried outwards along the surface by reason of these surface diffusing motions. If the liquid is one which spreads stably, then the spread film has a lower potential energy than the drop, so that the molecules which have left the drop to form a film stick to the surface; the surface diffusing motions go on continually underneath the drop, and the oil molecules which spread first are continually being pushed out further by the surface pressure of those just leaving the drop. If the liquid is a non-spreading one, a few molecules may diffuse out along the surface a little way, but being less mobile on the surface than in the drop, they will soon return to the drop, and will not stick to the surface.

The spreading of some liquids, for example, partially oxidised cubel, into a thin unstable sheet showing interference colors is probably due to the motions of the molecules of the oil, diffusing in contact with the water, transmitting the outward movement the molecules to the upper layers of the oil by viscosity. These are the duplex films of Marbine. As soon as the whole surface is covered, however, this film begins on to form a monomolecular film in equilibrium with visible drops of the liquid. Volatile substances spread in a similar

pressure, but equilibrium is never reached, since the drops and particularly the film are evaporating rapidly, and there is constant motion from the vapored liquid to make up losses from the film.

I

The film balance used for determining the drop in surface tension, otherwise known as the surface pressure, may be one of two types: the horizontal balance, first developed by Langmuir [5] or the Wilhelmy balance[6]. These differ in principle and each has its advantages. For measurement of low surface pressures, a sensitive balance is required, sensitive to pressure differences of 0.01 dynes /cm. or less. Either of the above balances may be used -- or both simultaneously. A balance of the Langmuir-Adam type was available at the beginning of the study, but was of a rather low sensitivity -- of the order of 0.1 dynes /cm. A Wilhelmy balance was also constructed so that the two balances could be used simultaneously for measurements of the surface pressure.

(A) HORIZONTAL FILM BALANCE

In order to understand the manner in which the horizontal film balance functions a brief account of its theory is necessary. Figure 1 illustrates the manner in which measurements are made.

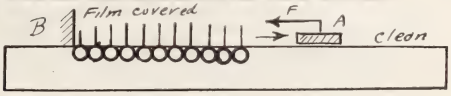


Figure 1

A light floating strip A bounds the film at one end; there is an area of clean water surface to the right of A. The area available for the film can be varied by

moving the heavy barrier B at the other end of the film. The light float A is connected with some instrument for measuring the natural force per centimeter [illegible] exerted on it by the film. This force is known as the surface pressure.

The connection between the surface pressure of the film and the surface tension of the film covered surface is also very simple. Surface tension is the free energy per unit area of the surface, or the work which must be done to increase the area of the surface by one square centimeter. If the floating barrier l is displaced a small distance dx to the right, then the work done on it by the surface pressure F is $Fldx$ where l is the length of the float. But if γ is the surface tension of the clean water surface and γ' that of the film covered surface, we must ldx by the energy γ the work we get by use of the energy γ', and the work done must be $(\gamma-\gamma')ldx$. These two expressions for the work done must be equal, hence $F=\gamma-\gamma'$. The surface pressure is therefore equal to the diminution of the surface tension of the water covered by the film. Harkins has shown that the horizontal force on the float is independent of the tilt of the float and of the way the liquid surface wets the float (6).

1. Concrete measurements with the horizontal balance.

The essential points requiring attention for accuracy are as follows:

(a) the barriers used to confine the film must be proof against leakage, under any surface pressure,

and must be heavy enough not to move under the surface pressure;

(b) leakage of the film past the ends of the light floating barrier must be prevented in such a way as to interfere as little as possible with the sensitivity of the measuring instrument;

(c) sufficiently sensitive means of measuring the force must be provided;

(d) all the apparatus must be as clean as possible and means must be provided for measuring the amount of contamination of the surface which is always present to some extent;

(e) an accurate means of measuring the amount of film-forming substance put on the surface must be available;

(f) the solvents for dissolving the spreading substance and the underlying liquid both must be free from all appreciable traces of film forming materials;

(g) the whole of the apparatus should be housed to ensure uniform temperature, and, if surface potential measurements are undertaken simultaneously, the balance should be shielded against electrical disturbances.

2. The Nideal balance.

The Nideal balance available at the beginning of the study is shown diagrammatically in Figure 2. Its construction was based on a design by Nikol (7). There is a trough in three levels across (not shown), filled to the brim with water; an instrument with one torsion

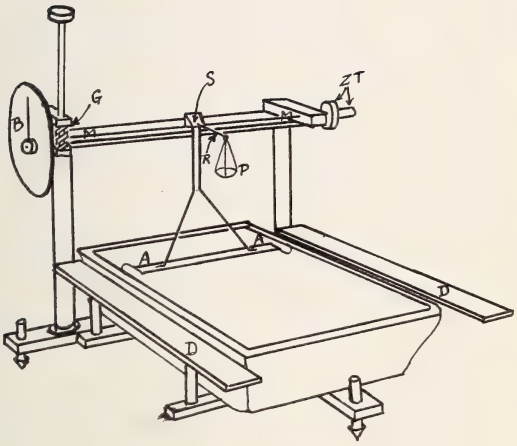


Figure 2.

Diagram of Rideal Balance.

wire for measuring the torsional pressure, placed toward one end of the trough with approximately 10 cm. of clean water surface behind it, and a considerable length of water surface in front of the instrument, on which the film is spread. Glass boundaries about 1 cm. wide, 2-3 mm. thick, 10 cm. long, mounted with a paraffin wax, are used to clean the surface in front of and behind the float [illegible], and are also used to compress the film.

The trough is a paraffined disk having a level ground edge which is waxed flat and will provide free horizontal motion. The float is a mica vane [illegible] attached to the torsion wire (W) by means of the Y-shaped stirrup made of duraluminum and is shorter than the width of the trough by about 2 to 3 mm. Leaks are prevented around the ends of the float by means of vaseline rubber or threads which are fastened to the float and the edge of the trough in the form of a U.

A mirror, attached to the stirrup just above the torsion wire by means of a small needle N, is used with a light and scale arrangement to render visible deflections of the float. At zero pressure on the float the torsion and zero twist of the torsion wire can be adjusted by the screw arrangement S2 on the right hand side of the head.

The instrument is used as a null instrument. With the pressure on the float transmitted to the torsion wire to produce a torque, indicated by the mirror. The torque is compensated by twisting the wire to move the

float back to zero position by means of the worm gear arrangement on the left side of the stand. The torque so produced is measured on the scale D and is a measure of surface pressure. The scale is previously calibrated by placing weights in the scale pan C and determining the total movement to bring the float back to zero. The pan is supported on the back of the end of the arm A which is in turn attached to the torque tube B and with the torsion wire and at right angles to the stirrup. A consideration of the data about the torsion wire with weights in the pan makes it possible to obtain a calibration curve for the torsion wire used in dynes / cm. on the float. One half of the thread of the ends of the float is considered as being part of the float. The position of the bubble for surrounding the film relative to the float is determined by the value b set vertical to the trough. The rules are graduated in millimeters and the area of the film can be determined with an accuracy of about one percent.

Calibration of the wire indicated that one scale division was equal to 0.40 dynes / cm. Hoke's law was obeyed over the range of 10 scale units for one meter.

. At it time of the ideal balance.

Although the ideal balance would fail for measurements of higher surface pressures, from 1 to 20 dynes / cm. and for development of technique in surface film studies it answered admirably the purpose.

The lack of a suitable device to bring the

[illegible] to zero and to hold it there while the surface was being cleaned around the cone to ensure that the surface was disturbed. This discouraged the use of a flame heated [illegible] to obtain maximum sensitivity.

The mirror attached to the stirrup just above the level of the steady wire moved through the same angle as the flow wire relative to the rims. With small increments of pressure this angle was small and deflection more than could be read accurately.

The threads attached directly to the float and sides of the trough were frictionless. Violent surface disturbances caused the threads to break loose from the sides and accumulated frictional effects and repetition of experiments. The direct attachment of the threads to the float and trough divided the surface into two separate sections each of which had to be cleaned. The section behind the float was too short to permit sufficient clearance for complete removal of surface pressure to be noted.

Manual operation of the barriers made it difficult to control the temperature and to shield the surface [illegible] alcohol [illegible]. Motion picture records of the films in an improved feature but had to be ignored. In practice, a speed setting of 2 cm. of the barrier which gave the maximum decrease in area desired for each pressure reading, was difficult to reproduce manually.

4. The new horizontal film balance.

A new horizontal film balance was designed by the writer to overcome these limitations and for the

[illegible] measurements of low pressures [illegible] are usually in evidence. The design of the [illegible] based on the Kidd balance and incorporates what the writer considers the best features of the balances described by Hopkins (8), Adam (5), Bergwin (3) and Harland (10), with original modifications. It has been employed so as to allow the simultaneous use of a [illegible] balance and the simultaneous measurement of surface potentials.

The head or measuring device of the balance is shown in Figure 2. The [illegible] is a [illegible] cm. long. The main part [illegible] of rigid [illegible] to the [illegible] of Figure 2. There are [illegible] attached to arms of unequal length, 2 cm. and 6.2 cm. [illegible] and [illegible] 2 cm. long. The other end of each ribbon is attached to the foot of a brass [illegible], which [illegible] the upper end in the main [illegible] of the head. When the head is in position above the trough the foot rests simply on the sides of the trough, held there by the action of two small wire [illegible] concealed in the foot. The foot is partly cut away so as to allow part of it to extend half the width of the gold ribbon into the water and thus prevent leakage of the film past the ribbon and foot. The ribbons are in a figure 8 shape, lie parallel to the sides of the trough, held in the water and [illegible] out and pass directly back to the foot. Thus pressure on the [illegible] evenly distributes the 8 shape of the ribbons, causes no change in the area of the film due to the movement of the ribbons and the

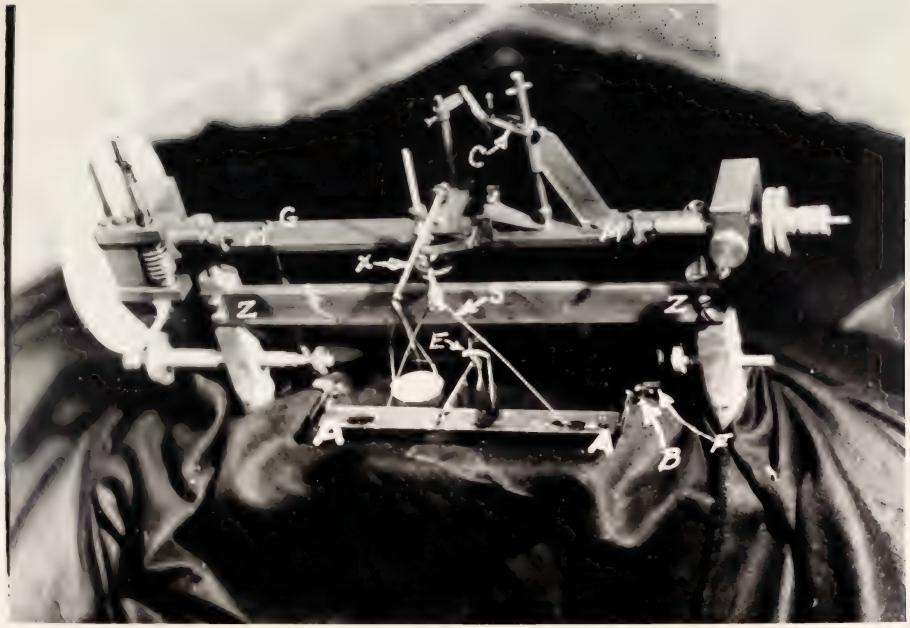


Figure 3. Head of Horizontal Balance

ribbons need not be considered when the grammes per unit length of the float is calculated.

The float is attached by means of the stirrup S to the upper torsion wire JK. The stirrup is extended past the wire in the form of a screw which may be fitted with a nut permits adjustment of the centre of gravity of the float and allows it to be balanced. The long arm L, 4.60 cm., which is perpendicular to the plane of the stirrup is provided with a hook from which to suspend the pan used in the calibration of the torsion wire. The pan is counterbalanced by extending this arm beyond the torsion wire and providing it with a screw nut and adjustment weight.

The torsion wire JK, 0.127 mm. in diameter, is of phosphor-bronze. Its tension may be adjusted by the screw arrangement used with the Abbot balance, a nut which forms a threaded rod through a hole in which it may be turned only if a set screw is loosened. The screw head and scale of the Abbot balance is used to turn the wire and measure the resultant torque, but the scale is fitted with a vernier pointer which permits readings to 1/10 of a scale division (0.2 degrees).

The stirrup can be cleared to zero position by the device C pictured in more detail in the diagram figure 3. By raising the rod C until it is just out of the tiny slot in its bushing BU, then turning it through a small angle the stirrup is first leveled, preventing forward and backward movement of the float, and is then

clamped by the small pliers PL, thus holding the float securely in a fixed position. The mechanism when reversed releases first the pliers and then the leveling device.

The mirror R is carried in a very light holder on a second torsion wire very close to the surface and behind the float. The torsion wire, 0.08 mm. in diameter, acts as a restraining spring for the mirror and may be adjusted for tension and torque by the same method as the mirror wire. A short tail extending down from the back of the mirror and nearly touching the surface is joined at its end by a short piece of silver wire, 1 mil in diameter, to the center of a small V shaped aluminum beam attached to the float, and which extends out over the surface beyond the mirror. Thus any pressure exerted on the float from the VW side of the trough and causing it to move will pull the mirror through an angle of approximately twice that the angle through which the float itself moves. The tail of the mirror, extended upwards above the mirror and bent at right angles, carries a small sheet of metal which damps movements of the mirror.

Extra rigidity is given to the frame of the head by the additional cross piece CB. The two vertical posts which support the head simply fit into two holes provided in corresponding supports which are part of the base, Figure 5, H. The head can therefore be easily removed from its position on the trough for cleaning and other purposes without the loosening of clamps or other devices. It can also be raised bringing the float

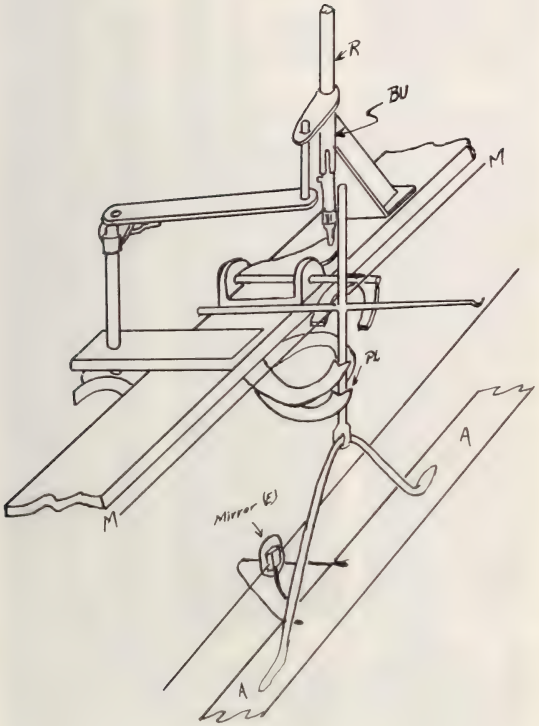


Figure 4.

Zero release and clamping mechanism.

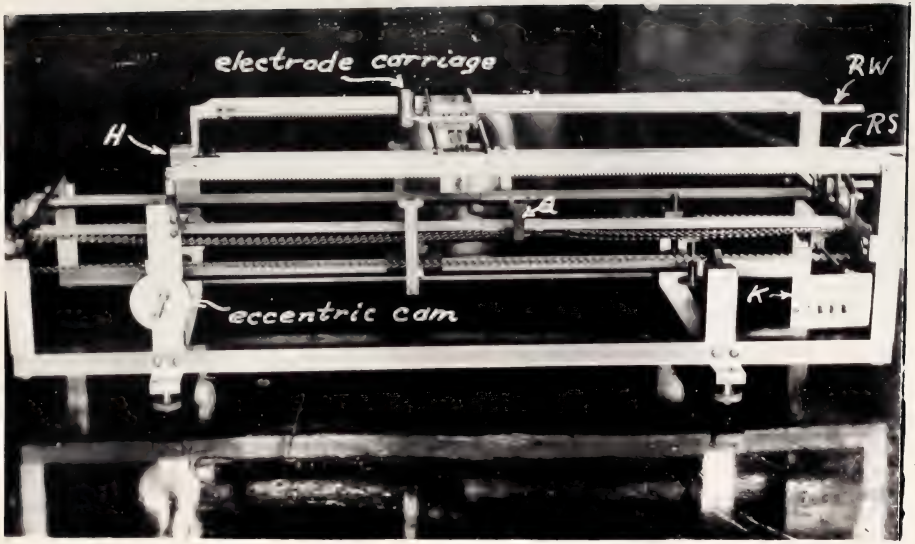


Figure 5. Base of Horizontal Balance

[illegible] the surface to alter the passage of [illegible] across the surface thus exposing it always of [illegible] time. The mechanism for raising and lowering the surface load is a cam device with two eccentric wheels on a common axle, (see figure 6).

The trough (60 X 10 X 2 cm.), made of cast [illegible], is cooled [illegible] and to insulate it [illegible] electrically. The sides and bottom of the inside of the trough are machined to a smooth finish, the width of the trough being exactly 10.00 cm. A machined [illegible] of copper of the same size as the trough is attached to its bottom. Water can be circulated through this [illegible] in order to [illegible]. The trough is surrounded by [illegible] which [illegible] the [illegible] [illegible] the [illegible] [illegible], figure 5, 1.

The film is compressed by moving the paraffined glass slide J (figure 6) over the water surface, which is done with the aid of a long screw [illegible] screw. One turn of the screw moves the slide forward 1.27 cm. and the counter, figure 5 2, is used to record the number of turns made with an accuracy of 1/20 turn, or 0.063 cm.

The weighing mechanism consists of two figure 6, two [illegible] rods, ridge supports for the fingers, a toothed wheel and chain for driving the fingers, and a spherical [illegible] of mercury (shown in figure 7) which makes it possible to turn both rods of the same

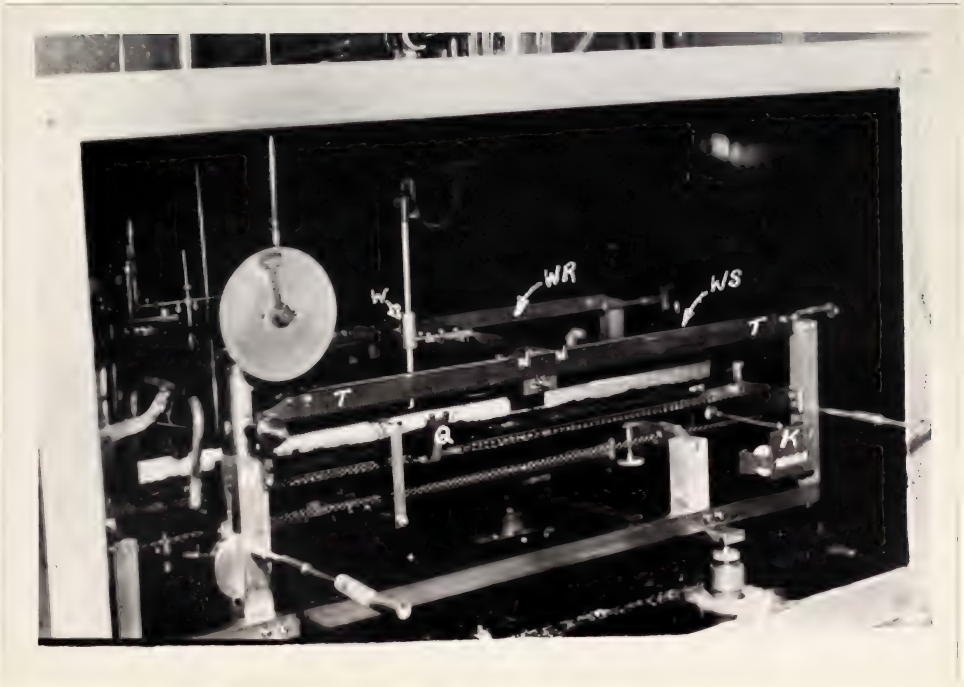


Figure 6. Horizontal Balance ready for operation

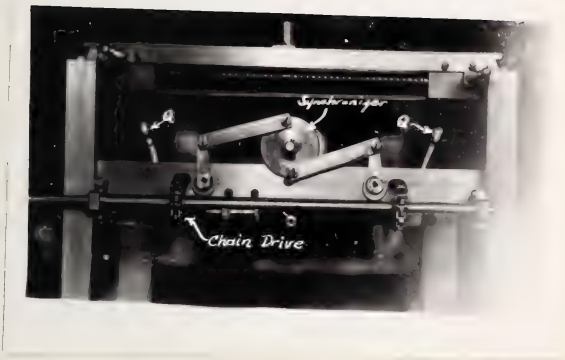


Figure 7. Sweeper sychronizing device

tire and so lower or raise the fingers together. With the arrangement it is possible not only to clean the whole of the outer surface from the point to which the finger is raised but also to remove or deposit glass slides on the corresponding mechanism figure.

The balance is also provided with a carriage, figure 5, B, for the placement and manipulation of small accessories. The carriage rests on two parallel rails running at right angles to the length of the trough and some distance above it. The carriage can be driven across the trough by turning the long rod C which drives the carriage along by means of two ninety degree gear wheels and a screw. The rails and carriage can then be moved parallel to the balance by turning the long shaft S which actuates a pinned pinion to move along the supporting racks R. This arrangement permits the complete exploration of the surface by the electrode.

The whole balance is enclosed in a double-walled outer case with doors provided at the mirror end for the light to enter and leave, and with a double-walled flexible front, figure 6. The shafts which are used to actuate the carriage of the balance are fitted with an ebonite plug which may be in the box and the flexible front. A small door in the front of the box permits ready access to the balance and is used for introducing the electrodes and material.

The balance itself is set on four projecting steel rods from a large (2 X 3 X 2½ ft.) concrete block

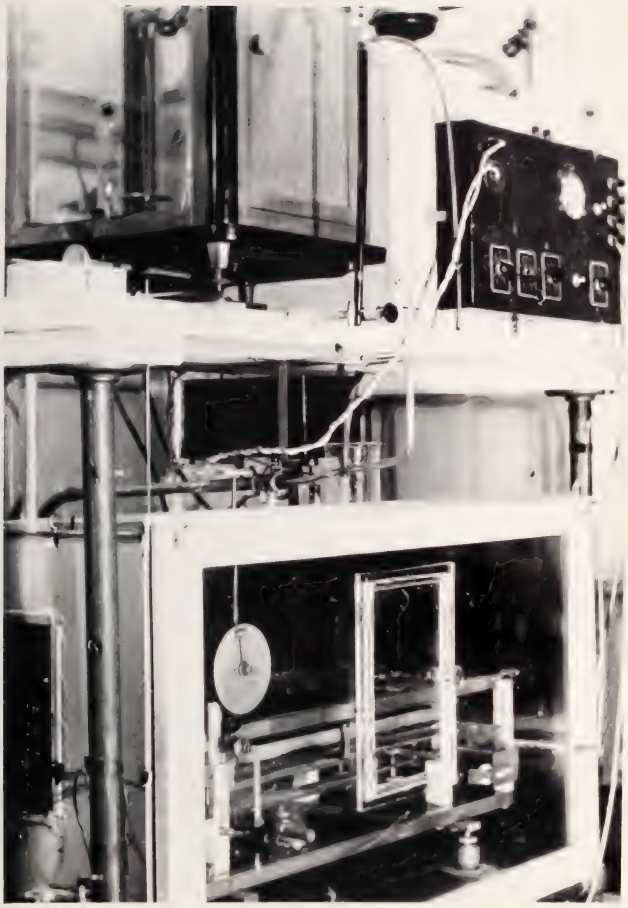


Figure 8.

Short range view of complete apparatus assembly

rich in iron rests on two inches of sand on a concrete floor. The sand prevents vibration and the concrete provides a steady and firm support for the balance. The legs pass through suitable holes in the copper box without touching; the copper box is supported separately by a one inch pipe frame screwed to the floor.

Water from a reservoir of capacity 45 litres, and maintained at a constant temperature by means of a benzene- mercury thermoregulator is passed through the walls of the copper tank and the bottom of the trough by gravity flow and then returned to the reservoir by means of a small centrifugal pump. The reservoir sits above the horizontal balance on a wooden platform supported by the same stout as the copper tank. It can be seen in figure 8 behind the Wilhelmy balance. The control panel of the surface potential apparatus is fastened to the front of it. Figure 9 is a view of the interior of the box with the balance in position.

(B) THE WILHELMY BALANCE.

The Wilhelmy method of determining surface tensions consists in measuring directly by means of a balance the pull of the surface of the liquid on its perimeter or a body such as a vertical microscope slide partially immersed in the liquid (8). If ω is the width of the slide and t is its thickness, its perimeter is $2(t+\omega)$. Then if φ is the angle of contact and γ is the surface tension, the vertical force due to the pull of the surface

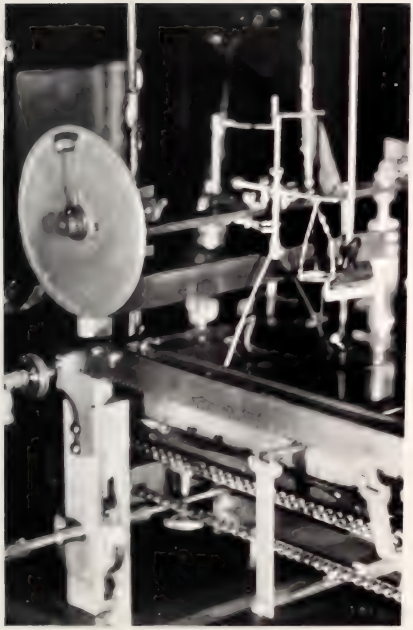


Figure 9.
View of head of balance
in position for operating

tension in the slide is $2\pi(t+\omega)\cos\varphi$. Let the force due to tension, equal to the weight of the entire slide, be $-g\rho l t\omega$ where g is the acceleration of gravity, ρ is the density of the liquid, and l is the depth of immersion of the slide. If W is the weight of the free air slide and G is the weight of the entire slide, the following equation holds at equilibrium

$$g\Phi = gW + 2\pi(t+\omega)\cos\varphi - g\rho l t\omega \qquad (1)$$

In case where $\varphi = 0$ and Φ in equation (1) is a maximum

$$\pi = \frac{g(G - W + \rho l t\omega)}{2(t+\omega)}$$

the type which rests on l, the depth of immersion, may be altered to be determinate for any load W which the horizontal component of the slide tension (the value of which is $l = 0$) and that determinate for which accordance is found, the slide must be made of the proper dimensions of the liquid.

The method just described may be modified [illegible] of the balance of the slide. [illegible] and unknown [illegible] the method is detail [illegible].

[illegible] of the [illegible] slide is [illegible] used for the [illegible] balance. In [illegible] slide is [illegible] shows the [illegible], and [illegible] [illegible] is used [illegible] the [illegible] of the [illegible]. [illegible] [illegible] slide is [illegible] below [illegible] and [illegible] in the [illegible] of the [illegible]. [illegible] [illegible] slide [illegible] and [illegible] [illegible] of the [illegible].

The deflection of the beam of the balance is read on a scale mounted about six feet in front of the balance by means of a telescope focused on a small mirror mounted on the beam of the balance just to the rear of the central knife edge. The zero mark on the scale is then visible in the water surface in glass. The two ends of a rule on the water surface below the surface level, so the glass rises and falls as the beam of the balance.

From equation (1) we may write, for $\varphi = 0$

$$g \Delta G = z(t+w)\Delta\gamma - g\rho a l t w \qquad (3)$$

Thus if the weight G is held constant ($\Delta G = 0$), the change in surface tension $\Delta\gamma$ will be proportional to the change in the depth of immersion of the slide Δl, or small deflection of the beam Δl is proportional to ΔS, the change in the reading of the scale viewed through the telescope. So that we have

$$g \Delta G = z(t+w)\Delta\gamma - k \Delta S \qquad (4)$$

in which k is the factor of proportionality.

The calibration of the balance is simple in determining the value of k by adding deflecting on the balance beam ΔS for a read on the balance with the slide immersed in a clean water surface ($\Delta\gamma = 0$). So we have the relation

$$k = -\frac{g \Delta G}{\Delta S} \qquad (5)$$

The reduction of the surface tension, $\Delta\gamma$, or surface pressure, caused by the presence of the

ΔS

$\Delta G = 0$

$\Delta \gamma$

$$\Delta \gamma = \frac{k \Delta S}{2(t+\omega)}$$

4. [illegible].

(a) [illegible] in surface potential.

The surface potential change (ΔV) produced by spread monomolecular layers may be measured in the following manner. The air above the air-liquid boundry is made locally conductive by means of a radioactive polonium preparation. Simply make the liquid be a colonel electrode. The potential difference between the colonel electrode and the polonium preparation depends on the local conditions in the air ionic boundry. The presence of a monolayer gives a change in the surface potential, which can be measured with a potentiometer and a type of electrometer. This method of measuring was originated by Guyot and Gaudin (10) and modified by Adam and Dunning (11) and others, (10), (12).

The electrical circuit devised for use in connection with the polonium and colonel electrodes is based on that of Adam and Dunning, who used a triode electrometer (11). The circuit of Le Gaillou and Jouan (13), involving the use of an FP 54 tube developed especially for amplification of small direct currents, constitutes an improvement and is used. It is shown in Figure 10.

In order to understand how the circuit functions consider first the portion to the right of the dotted line. For simplicity it can be regarded as a Wheatstone bridge in which R5 and R7 form two of the resistance arms, and the filament-to-cylinder resistance and filament-to-grid-charge-grid resistance form the other arms. The resistance

STABILIZED ONE-TUBE CIRCUIT FOR MEASURING SURFACE POTENTIALS

(Dubridge and Brown)

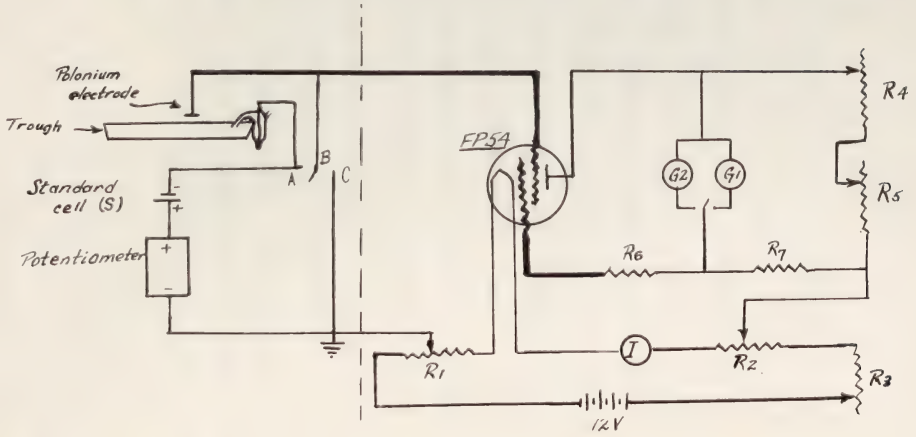


Figure 10.

R_6 may be considered a part of the tube resistance. A condition that the galvanometer current be zero is

$$R_6 I_p = R_7 I_s$$

In order that this condition remain satisfied when the battery voltage changes, or the filament emission changes for any reason, it is further necessary that

$$\frac{dI_p}{dI_f} = \frac{R_6}{R_7} \frac{dI_s}{dI_f}$$

For these conditions to be satisfied in general, it would be necessary, for the I_p versus I_f and I_s versus I_f curves to be straight lines intersecting at a common point on the I_f axis. The tube do not have this characteristic, but over short ranges the tangents to the I_p and I_s curves do satisfy this condition, and it is possible to adjust the resistances in the circuit so that this can be made to occur at approximately the rated filament current.

The advantage of this ingenious circuit is that any change in the filament emission can be variables in battery voltage or sensitivities of the filament is compensated. Also, short period fluctuations in the filament emission are balanced out. The high input resistance of the 1274 tube, of the order of 10^{10} ohms renders the tube and this circuit very useful for the measurement of surface potentials.

The procedure in balancing the circuit is as follows: The grid is first connected to electron the switch K. Then with the narrow galvanometer G2 in the circuit, that is with the switch in position K2 and R3 adjusted so that the galvanometer reads zero when I_f is near its rated value, I_f is slowly varied by means of the rheostat

12. With the galvanometer connected so that a positive deflection is caused by a decrease in the plate current, the deflection should pass through a maximum value for some value of I_f.

If the value I_f for a maximum is not within a few percent of the rated value for the tube, the adjustment of V_1 and R will bring the balance point to a different value of I_f. The adjustment is readily made with the sensitive galvanometer G1 in the circuit.

Having obtained a maximum in the galvanometer deflection corresponding to the rated filament current, it is only necessary to change R_2 and R_4 to make the maximum coincide with a zero deflection of the galvanometer.

The switch S_2 is then closed and the potential of the standard cell E is balanced by varying the potential on the potentiometer P, again no deflection on the galvanometer G being permissible. Now by varying the potential on G so that the grid acquires a known potential a deflection on G is obtained, corresponding to the applied grid potential V_1 minus V_2. Thus the galvanometer deflections are calibrated in terms of applied grid potential.

The switch at D may now be opened bringing the platinum and colored electrodes into the circuit. With a clean water surface under the platinum electrode, and S_1 set to contact 4, the pair assumes a potential V_1, noted by the deflection on G. S_1 is then placed on the water surface and a new reading of the potential is noted V_2. Then $\Delta V = V_1 - V_2$ = change in potential due to the presence of the monolayer.

(II)

The resistances $R_1, R_2, R_3, R_4, R_5, R_6, R_7$

and the millivoltmeter I will presently discuss are mounted in a metal box which can be seen in Figure 6, attached to the side of the reservoir box. The bottom of this control box is shielded by a metal screen. Both the box and screen are grounded. The tube is placed in a separate metal box with removable silver discs above the centre of the thermostat box. The tube box is fastened to the bottom of the wooden platform which supports the Pilbeby Balance. The switch SW is also contained in the tube box and is made of copper with polystyrene insulation. Small convection trough containing Anhydrone powder is placed in the tube box to ensure a dry atmosphere. The grid lead is short and passes directly through a copper tube shield to within the thermostat corner box. A spring coil of soft copper wire connects the grid lead to the middle electrode. The tube box and the thermostat box are both grounded.

The potentiometer (type K3) the coarse galvanometer, standard cell and switches are all placed on a metal screen covered table next to the horizontal balance and all circuit leads are shielded. A cable covered wire is used for leads in all cases where shielding is necessary. The batteries are contained in a heavy wooden box with a metal top, cutting down temperature fluctuation and unstable electrical disturbances which contribute to drifts of small voltages.

The coarse galvanometer is a Leeds and Northrup

1. Preparation of the Calcium Electrode.

The [illegible] calcium amalgam electrode was prepared by putting a small [illegible] silver wire to be a solution of mainly acidic calcium chloride [illegible] results from the decomposition of which can be obtained [illegible] It is the second [illegible] of the decomposition product having a half-life period of 1.8 days.

It was obtained by [illegible] calcium needles [illegible] old), of purity [illegible] of hot dil. [illegible] for an accurately one-half hour. The [illegible] was washed, dipped in [illegible] dil. [illegible] The clean [illegible] for use from [illegible] the silver wire to be used and [illegible] placed out on the [illegible].

A second electrode was prepared like the first one [illegible] of activity. It was prepared in the same way as the first according [illegible] procedure for obtaining the calcium chloride solution was modified according to a method described by Kolthoff (10), in order to obtain a purer one [illegible] calcium content.

Since the [illegible] of calcium on silver is determined with [illegible] the solution contains traces of a [illegible] oxidation e.m.f. [illegible] recovery. These are corrected by the following procedure.

The radon emanation [illegible] and treated with dilute acid [illegible] precipitation is carried out

with ammonium hydroxide to eliminate almost all of the iron and copper, if present. The precipitate is dissolved in dilute hydrochloric acid and a solution of ammonium in 20 percent hydrochloric acid is added. The resulting precipitate is digested and then filtered off. The filtrate is then diluted and a thin silver plate of approximately 3 sq. cm. in area is rotated in the solution for at least 12 hours. Most of the polonium is deposited on this foil which is then dissolved in hot concentrated nitric acid. The silver is precipitated by addition of hydrochloric acid. The filtrate is evaporated to dryness and the residue dissolved in a minimum quantity of dilute hydrochloric acid (approximately 0.5 N). The silver electrode plate is revolved in this solution for 12 hours with the consequent precipitation of the pure polonium on the electrode plate.

III

An extremely simple and useful technique for the detection and identification of proteins has been developed by T.F.Schaefer (71). A colored film of indicator oil is spread to cover about half the free surface of the water in a trough so there is no surface pressure. Indicator oil is prepared by emulsifying mineral oil until a drop of it when placed on the surface of water spreads to give a visible grey film of uniform thickness(1 molec film). Some of the protein is placed in the centre of the patch of indicator oil, either as a drop of solution, or a tiny protein fragment. The

protein spreads out against the indicator oil, the outline made visible as a dendritic pattern, known as the external spreading pattern. If a small drop of indicator oil is touched to the centre of the protein monolayer, the protein is then seen to the oil which gives a characteristic internal spreading pattern.

The expansion pattern for pepsin is shown in Figure 11. The internal pattern is star-like; usually the star has five points. Its typical external pattern is smooth and circular. If several more drops of the oil were touched to the surface of the pepsin monolayer between the external and internal pattern boundaries were (more could be formed, showing that the star is characteristic of the protein and that it exhibits no memory of its point of origin on the surface.

The apparatus required for a study of protein patterns includes:

(1) a trough similar to that used in other monolayer work but having a black bottom,

(2) waxed glass barriers for confining the film,

(3) indicator oil,

(4) platinum wire for depositing the indicator oil on the surface, and

(5) photographic apparatus for taking pictures of the patterns if these are desired.

IV [illegible]

The measurement of the area of surface covered per molecule of the barrier material necessitates the

Figure 11.

Expansion pattern for pepsin

Figure 12
Blodgett
Pipette

The pipettes constructed had a volume of the order of 1.10 ml. and in a series of determinations the average error for the volumes delivered were less than 0.3 percent.

the temperature difference to better than 1/2° degree. Runs are made on days when the temperature of the surrounding atmosphere and the underlying soil/ice are the same.

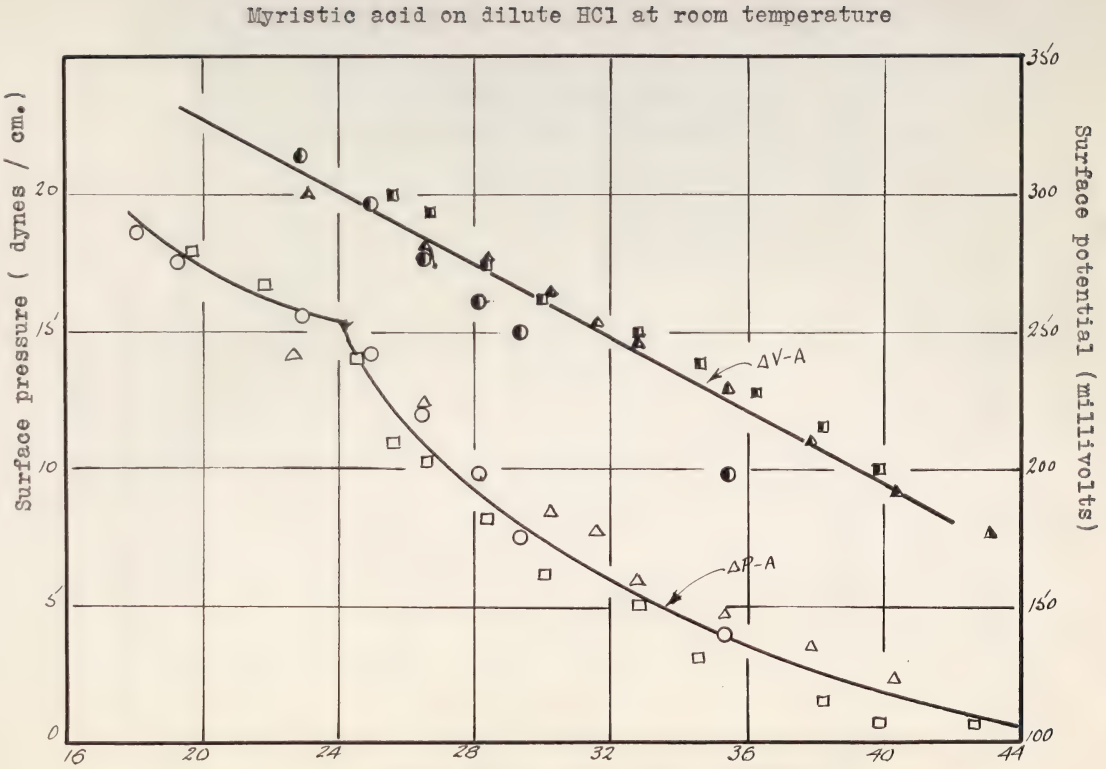
THE SURFACE BALANCE AND SURFACE POTENTIAL APPARATUS.

In order to test the surface potential apparatus and the Gideal film balance films of myristic acid were investigated. The experiments were carried out at room temperature on 0.01 N hydrochloric acid.

The results of three of the preliminary experiments are indicated in Figure 1. The curves when compared with those obtained by other investigators (4) (17) (16) indicate that the convergence are of the proper order. Deviations encountered can readily be accounted for by impurities in the water, error in the measurement of the quantity of acid placed on the surface (0.01 to 0.03 ml. measured with micropipettes) and leakage past the rubber strands.

The film balance was shielded from draughts and hence from large surface tension fluctuations by a covering of cardboard and a further experiment was carried out. The results are indicated in Figure 14. In the lower curve surface pressure is plotted against the area per molecule occupied by the film and in the upper curve the change in surface potential is plotted against the surface area per molecule.

The pressure-area curve shows good agreement with pressure-area curves recorded by Adam(3) but a difference in the area corresponding to the transition point is noted. The error may have been due to faulty


Myristic acid on dilute HCl at room temperature
Surface pressure (dynes / cm.)
Surface potential (millivolts)
ΔV-A
ΔP-A
Area per molecule (Sq. Angstroms)
0
5
10
15
20
16
20
24
28
32
36
40
44
100
150
200
250
300
350
Figure 13.

Myristic acid on 0.01 N HCl with the Rideal balance

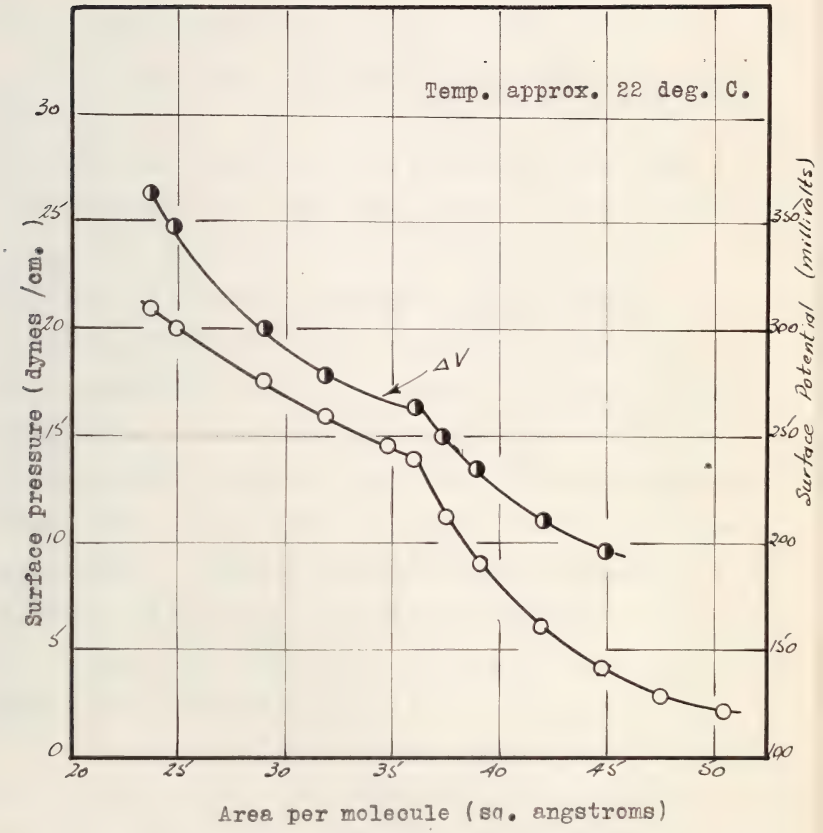


Figure 14.

movement of the reality of cyclotic acid placed on the surface or to a difference in the rate of compression. Thus Hasting and Hutting (16) report molecular areas for the transition point 25 percent lower than those reported by Adam and show that the curves obtained are affected by the rate of compression.

The surface potential curve shows a transition point at the same area as the surface pressure curve and agrees in range and shape with measurements reported by Goddeliefroi (15). Each value of the surface potential shown on the plot is the average of at least six readings taken at different positions on the surface. The successive readings showed no greater deviation than those reported by Harkins and Fischer in similar measurements (17). The surface potential results undertaken by Harkins and Fischer on hexadecyl alcohol films showed corresponding to the transition point but showed erratic measurements. However, it was found that a hysteresis effect due to the rate of compression can occur and it is thought that the results obtained in this work exhibit this phenomenon.

Surface potential measurements are valuable because they are related to the amount of orientation of the molecules on the surface and their values depend on the dipole moments of the film producing substances as well as on the number of molecules per unit area. It is also possible to test the homogeneity of the surface with surface potential apparatus and as

confirm the existence of more than one phase on the surface of the same wire.

Thus in Figure 14 at areas beyond 50 square centimetres the surface potential fluctuates slowly as the electrode explores the surface showing that more than one phase is present. The two phases in equilibrium at these areas are in fact the liquid and gaseous phases. The lack of sensitivity of the Nideal balance rendered it impossible to make surface pressure measurements at these areas.

II INVESTIGATIONS OF MYRISTIC ACID FILMS ON THE NIVERSITY BALANCE.

In Figure 15 are shown the results of a preliminary investigation with the Nideal balance. Myristic acid was spread from a 0.01 % chloroform acid solution contained in the Nideal balance trough. The myristic acid was delivered with a calibrated micropipette and the temperature was approximately 24 degrees Centigrade. This experiment represents the best of a series of four experiments with this balance and was carried out with the object of obtaining a plateau on the pressure-area curve. The curve obtained is in very good agreement with those obtained by Adam (3) at the same temperature and clearly demonstrates the applicability of the instrument.

Calibration of the balance indicated that a deflection of one scale division was equal to 0.041 dynes / cm., reproducible with an average error of 1.1% .

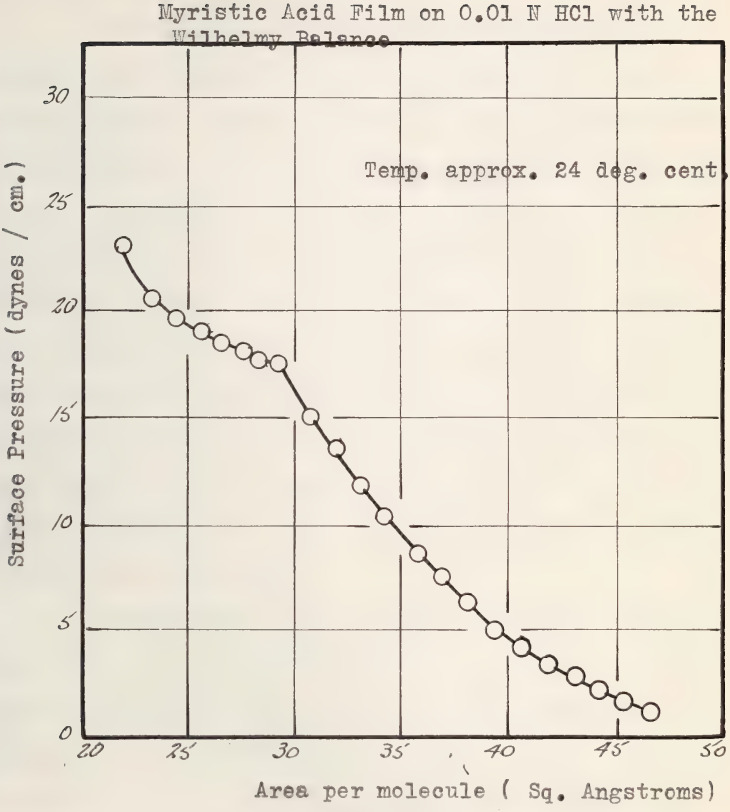

Myristic Acid Film on 0.01 N HCl with the Wilhelmy Balance
Temp. approx. 24 deg. cent.
Surface Pressure (dynes / cm.)
0
5
10
15
20
25
30
20
25
30
35
40
45
50
Area per molecule (Sq. Angstroms)

Figure 15.

However, experiments on gaseous films were not undertaken with this apparatus because of (1) limited size of the trough, (2) inability to measure small volumes of more dilute solutions accurately, (3) inability to control the temperature accurately.

The choice of balance for conversion to a Gilbert type balance was an unfortunate one. The mechanical device which released the beam of the balance for measurement was of the lifting type. Thus when the beam was released for measurement the dry glass slide was lowered into the solution instead of being raised out of the solution with a wet surface and the contact angle between the water and glass was not always zero. This error could have been minimized if the atmosphere around the surface had been saturated with respect to water vapor, but with normal compression of the film and the type of enclosure used a water saturated atmosphere was difficult to obtain.

Experiments with the Gilbert balance clearly showed the necessity for mechanical manipulation of the compression barrier inside a closed thermostat box the interior of which could be filled with a water saturated atmosphere. They further indicated that another modified balance should be used; one whose beam could be released without a lowering of the beam itself.

III MEASUREMENTS ON GAS ADSORBED FILMS AT THE SOLID-GAS INTERFACE ON AN ADSORBENT POSSESSING A LARGE SURFACE.

1. Spreading technique.

Various techniques are used for the measurement

of proteins. Hayes and Rideal (21) spread the film from solid protein attained by a quartz fiber method weighed on a microbalance before and after spreading; Carter (10) spreads protein fibre from a very dilute aqueous solution with a micropipette. This technique has been modified successfully by Davies (12) who adds from 1 to 2 parts per thousand of methanol -1 to aid spreading. Hall (19) uses Carter's technique but uses a concentrated solution of ammonium sulfate as an underlying liquid. Carter (10) and Langmuir and Schaefer (34) report that concentrated salt solutions as an underlying liquid aid in the spreading of protein monolayers. Langmuir (28) spreads the protein solution along the edge of a metal strip of length equal to the width of the trough. The spider of water presumably spreads the protein as a homogeneous and complete monolayer.

Carter's method is favored by most workers and was used in these experiments. Distilled water, pH 5.8, was used as an underlying liquid.

2. Results

The force-area and surface potential-area curves were measured for two fractions of zein protein obtained from the Department of Plant Science. The results are tabulated in Figure 16 and Figure 17 for the water soluble and salt soluble fractions respectively.

It should be noted that surface pressure and surface potential are plotted against total film area in both cases. It was desired to determine the general

PEA PROTEIN -- water soluble fraction

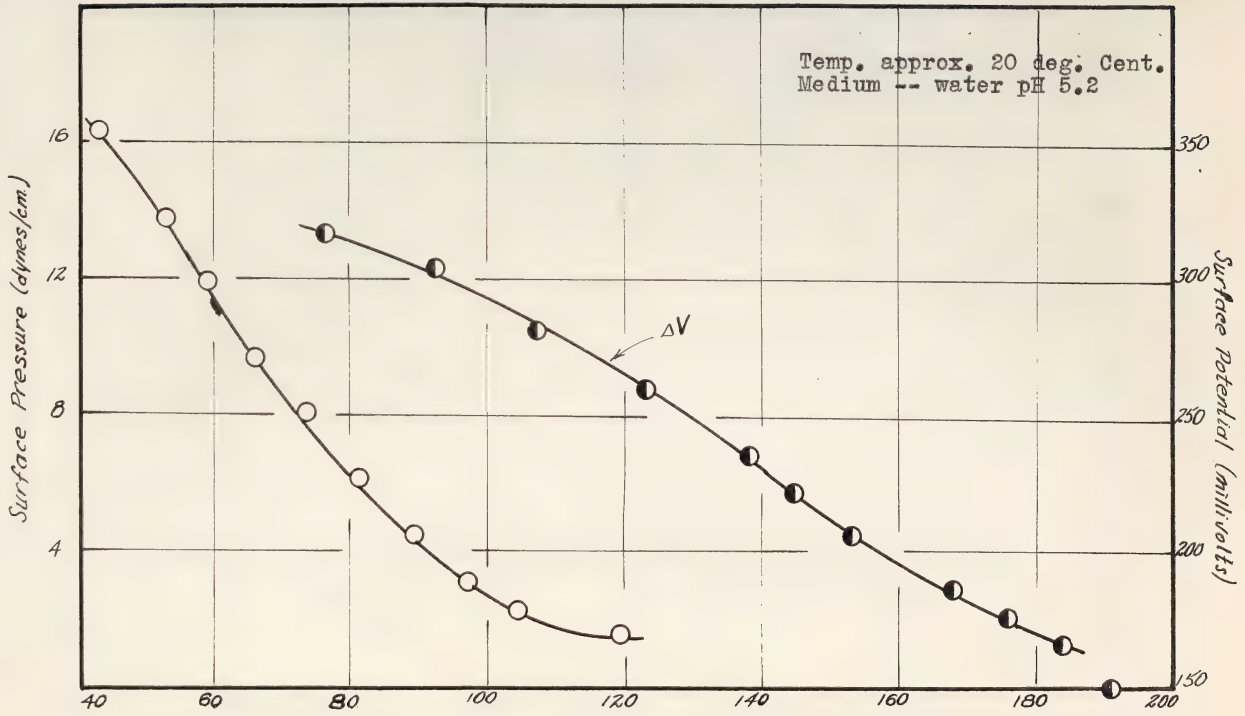
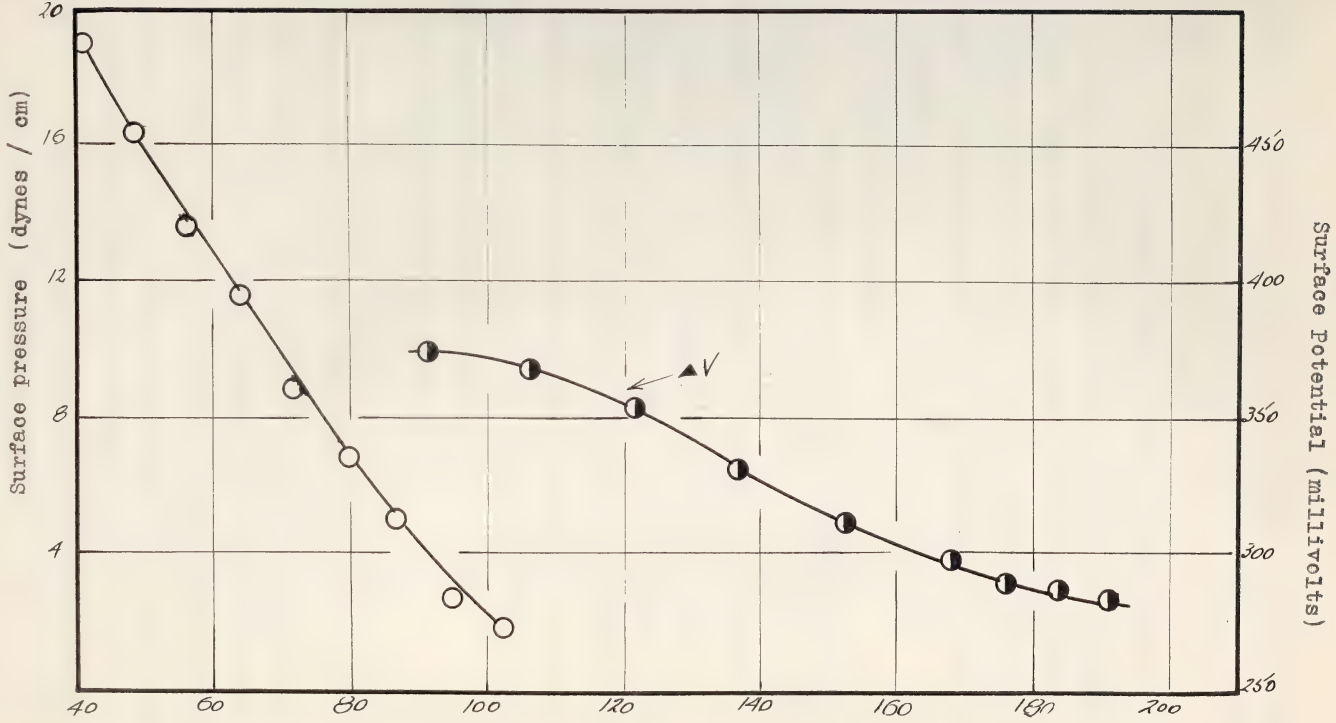


Figure 16.

PEA PROTEIN --salt soluble fraction



Area of the film in sq. cm.
Figure 17.

nature of the curves and, in particular, to see whether the film balance was capable of detecting pressure-area relations in the gaseous region.

The curves agree in their general character with protein curves as reported by Hall (20), Elliott [9] and others [21]. They report that generally above one dyne /cm. pressure the protein films exist as liquid films at room temperature and as compression becomes solid and elastic.

3. Expansion patterns.

The expansion pattern for a film of the water-soluble fraction of pea protein was one of the smooth circular type and indicates, according to Langmuir, that the monolayer is in a fluid stage at this pressure (between 0.5 and 1.0 dyne / cm.). The pattern produced by the salt soluble fraction was of the star-like form. Protein monolayers which produce patterns of the star-like form are in general of the type described by Langmuir and Adams as a gel structure.

4. Interpretation of Results.

From the surface potential measurements, which were made to much larger areas than it was possible to detect any surface pressures by the Wilhelmy balance, it would seem that a change of phase to the gaseous state occurs at areas of about 130 sq. cm. for both proteins. Surface potential measurements beyond this area changed quickly and fluctuation was evident.

The expansion pattern for the water soluble

protein fraction indicates that it is fluid at pressures of less than 1.0 dyne / cm. and it is likely that an area of about 90 sq. cm. for the film, corresponding to a surface pressure of about 4.0 to 5.0 dynes / cm. the film becomes solid and elastic. This is indicated by the change in shape of the surface potential curve. The salt soluble fraction is a gel at pressures as low as 0.5 to 1.0 dyne / cm. and probably would not become fluid until expansion to areas greater than 150 sq. cm. as indicated by the slope of the surface potential curve.

IV Measurements on Crystalline Acid Films with the Monomolecular Balance.

Three experiments were carried on crystalline acid films with the new instrument between using 0.000170 M, 0.00000 M and 0.000,044 M solutions of crystalline acid respectively on a 0.01 N hydrochloric acid substrate. A Mohr pipette of capacity 0.0216 ml. was used for measuring the quantity of solution placed on the surface each time and compression was slow and uniform.

Calibration of the balance indicated that 1 scale division was equal to 0.043 dynes / cm. and a rise in pressure of 0.004 dynes / cm. was observable.

The results for the 0.000,044 M solution are plotted in figure 10. These results were chosen for illustration because with the fullest extent of surface available, they indicate the nearest approach of the film to an ideal two dimensional gas. Surface pressure

Gaseous Myristic Acid Film on 0.01 N HCl

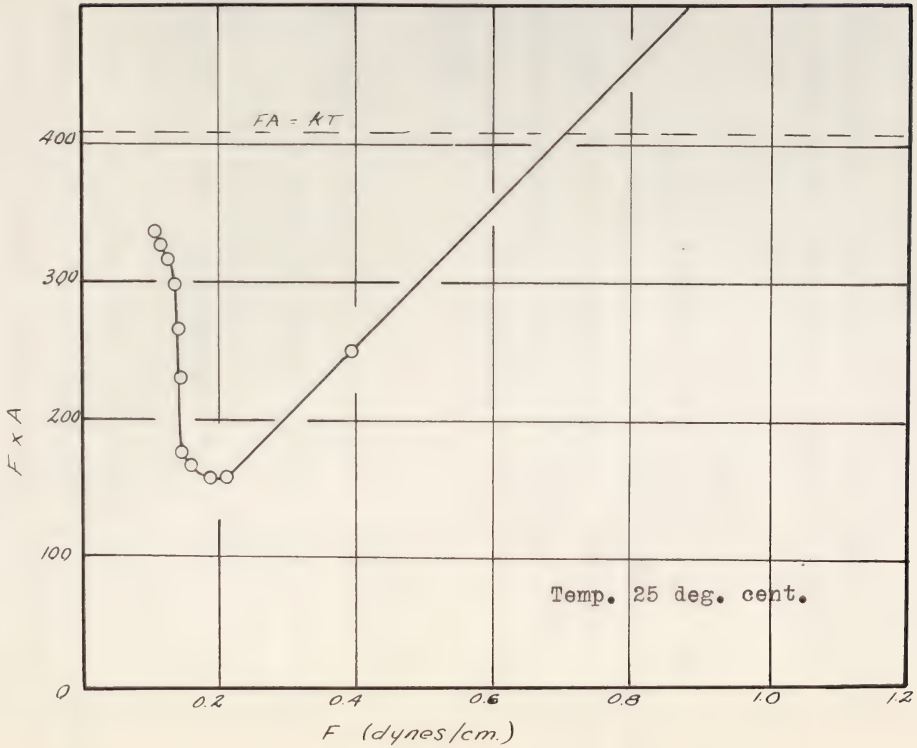


Figure 18

multiplied by area per molecule is plotted against surface pressure.

The resemblance to FV-I isotherms for two dimensional gases is clearly recognizable. The curve is similar to curves reported by Adam and Jessop for myristic acid under similar conditions (47). The curve shows that the pressure at large areas reaches the order of the theoretical perfect gas pressure, given by the equation

$F \times A = 1.372\ T$ in which

F is the surface pressure in dynes / cm.

A is the area per molecule in square angstroms.

The line $FA = 409$, corresponding to a temperature of 22.4 degrees centigrade is shown on the diagram. Extrapolation of the curve to zero surface pressure gives a value of 420.

V MEASUREMENTS ON THE ALBUMIN FILMS FOR WHICH HORIZONTAL BALANCE AND WILHELMY BALANCE WERE USED.

In order to further test the new horizontal balance and the Wilhelmy balance an experiment was carried out with egg albumin. Salt free egg white, by measuring surface pressure - area relations in the gaseous phase, to evaluate the molecular weight of egg albumin and obtained a value (44,000) in good agreement with molecular weight values by other methods. In the determination he used the Wilhelmy balance and a 2% percent solution of ammonium sulfate as an underlying solution. That the egg albumin is pure is indicated

by its expansion pattern shown in Figure 19. Both the internal and external shielding patterns are typical. The details of the method were duplicated in every known respect in this experiment and the results are indicated in Figures 20, 21, 22.

In Figure 20 are plotted values obtained for FA against F where surface pressures are low with the horizontal balance and the manometer is in a gaseous state. The best straight line through the points shown was calculated by the method of least squares. The resulting equation $FA = 0.988F + 0.061$ compares favorably with that of Bull, viz;

$$FA = 0.970F + 0.0554$$

The molecular weight for gas chloride calculated on the basis of the ideal gas laws is 92,300, a value within 10 percent of Bull's.

The Gilmont balance did not respond very well in this determination of low surface pressures but its range of application yielded values for high surface pressures, i.e. up to 45 dynes / cm. whereas the horizontal would require a longer diameter tension wire for such measurements.

In Figure 21 are plotted values of F against A for values of area smaller than 1.0 sq. meters per gm. of gas chloride, surface pressures up to nearly 25 dynes/cm. Only five points were obtained for this portion of the curve due to the fact that the area of the film was so small in this range of pressures that a decrease of area caused by a 2 to 3 mm. forward motion of the compression

Figure 19.

Expansion pattern for

egg albumin

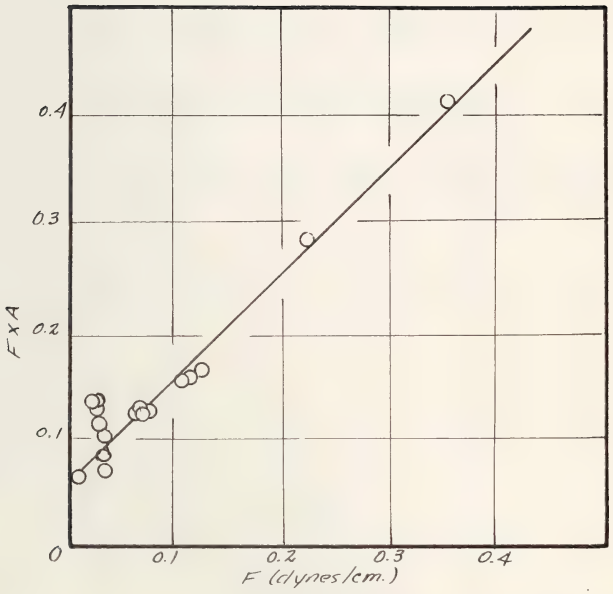


Figure 20.

FA-F curve for egg albumin with the horizontal balance

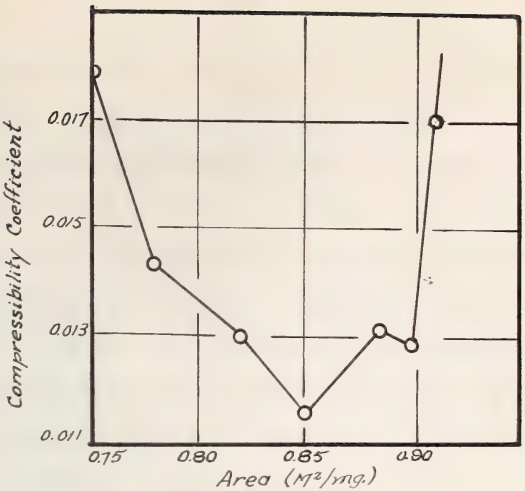


Figure 22.
Compressibility coefficient for egg albumin on Wilhelmy balance

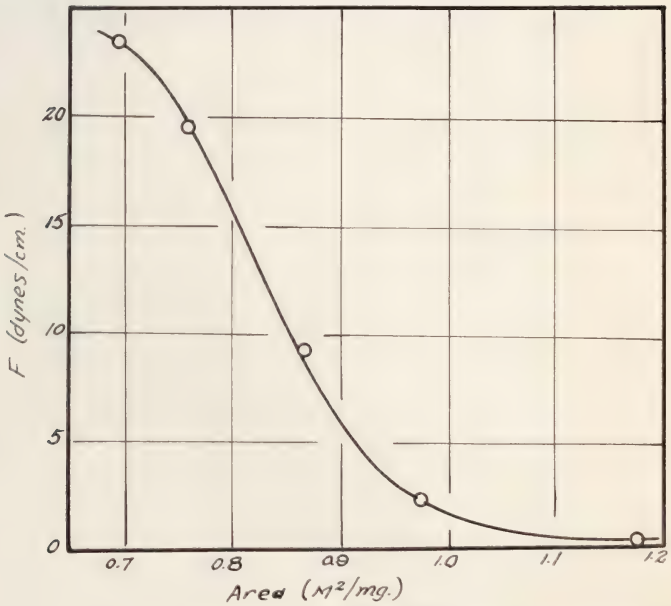


Figure 21.
Force-area curve for egg albumin on Wilhelmy balance

barrier decreased the area as much as 0.12 M^2/mg of protein. For more careful investigation of this portion of the curve more protein would be placed on the surface initially with the whole available trough surface resulting in a coverage of 1.[illegible] sq. m./ mg. of protein. The curve however fairly well agrees with that reported by Hull, and when compressibility of the protein as obtained by drawing tangents to the curve is plotted against area / mg. of protein (Figure 23) a curve very much the same as Hull's is obtained.

Hull reports a minimum compressibility coefficient occurring at 0.02 sq. m./mg. whereas in figure 23 the minimum occurs at 0.[illegible] sq. m. / mg.

Abstract

The Wilhelmy balance was used in preliminary studies of protein monolayers and its applicability has been tested with measurements on myristic acid monolayers. From the nature of the results obtained it was decided to design a new horizontal balance. The construction of this balance has been described in detail.

An apparatus for the determination of changes in surface potential has been developed and successfully used for surface potential measurements on myristic acid and pea protein films. The sensitivity and applicability of the instrument compare favorably with others developed for the same purpose (10), (11).

A Wilhelmy balance has been constructed for the purpose of measuring surface pressures simultaneously with the horizontal balance. It was tested by determining an area curve for myristic acid on the liquid state. Good results were obtained with this balance but were not reproducible. Difficulty is experienced in maintaining a zero contact angle between the water and the slide. For more sensitive measurements with this balance a thinner and wider slide might be used.

The high degree of precision required for surface pressure measurements of films in the gaseous region has been obtained by the use of the new horizontal balance. Its application in this respect has been shown in the results for gaseous myristic acid films which

correspond very closely to results reported by Adam and Jocelyn (27).

The molecular weight of egg albumin was determined from the surface pressure-area relation measured by the new horizontal film balance. Although only one experiment was made, a value of 40,200 for the molecular weight of the egg albumin was obtained, a result which compared favorably with that of Adam (22) who used the Wilhelmy balance. This clearly demonstrates that the new horizontal balance may be used to measure the molecular weights of proteins which form monolayers.

The use of expansion patterns to aid in the interpretation of surface pressure and potential measurements has been demonstrated. They can also be used to quickly determine the purity of a protein whose pattern is known. The pattern technique was used to indicate that the egg albumin, whose molecular weight was determined, was reasonably pure.

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